

## RESEARCH

# Genotype × Environment interaction for antioxidants and phytic acid contents in bread and durum wheat as influenced by climate

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Antioxidants prevent oxidative stress and exert positive health effects. However, phytic acid among them decreases micronutrients absorption, representing also antinutrient to human and non-ruminant animals. Fifteen bread wheat (*Triticum aestivum* L.) and 15 durum wheat (*Triticum durum* Desf.) genotypes were evaluated across six environments to determine contents of phytic acid (PA), inorganic P (P<sub>i</sub>), total yellow pigment, total soluble phenolic compounds, free protein sulfhydryl groups (PSH), and also phytic acid P/P<sub>i</sub> (P<sub>p</sub>/P<sub>i</sub>). The objective of this study was to quantify, for each trait the effects of environment, genotype, and their interaction; and the influence of climatic factors on the Genotype × Environment interaction (GEI) by the use of the factorial regression. GEI (P < 0.001) prevailed as source of variation over genotype (P < 0.001) in determining PA content in bread and durum wheat (44.3% and 34.7% of sum of squares-SS, respectively), PSH content in bread and durum wheat (27% and 28.4% of SS, respectively) and total soluble phenolic compounds content in durum wheat (35.5% of SS). The major contribution to the GEI represented climatic variables during stages of stem elongation for PA and phenolic compounds, and also flowering, fertilization, grain formation and grain filling for PSH. Total yellow pigment and P<sub>i</sub> contents in bread and durum wheat were predominantly determined by genotype (P < 0.001). Models of climatic variables proved to be efficient in the explanation of more than 92% of the SS of GEI for PA and antioxidants contents.

**Key words:** Antioxidants, climatic factors, Genotype × Environment interaction, phytic acid, *Triticum aestivum*, *Triticum durum*.

## INTRODUCTION

Common (bread) wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) are the most economically important wheat species, representing commodities with different technological quality as the result of breeding for milling and processing industry demands. The quality of wheat grain is influenced by genotype, environment and Genotype × Environment interaction (GEI). Understanding these effects is necessary to create strategy and goals of breeding for high yield and consistent quality traits tailored to the needs of the market (Vázquez et al., 2012).

Antioxidants represent compounds which inhibit the initiation or propagation of oxidative chain reactions initiated by the formation of reactive oxygen species (ROS), and have health benefits (Sivaci and Duman, 2014). Numerous studies have been conducted with the aim of phytochemical identification and quantification of the compounds in foods with an antioxidant activity, also unraveling their antioxidant capacity and role in the prevention of diseases associated with oxidative stress (Moore et al., 2005; Li et al., 2008; Gökmen et al., 2009). Yellow pigment is an essential factor of durum wheat end-use quality and represents quantitative trait with complex genetic background and also is influenced by environment. It is defined as the content of extracted carotenoids of the endosperm and is expressed as the content of β-carotene. Phenolic compounds, secondary metabolites in plants, have protective role against degenerative diseases-heart disease and cancer in which are involved ROS-superoxide anion O<sub>2</sub><sup>-</sup>, hydroxyl HO<sup>•</sup> and peroxy ROO<sup>•</sup> radicals, and also mixed N-oxygen species (RNS)-nitric oxide (NO<sup>•</sup>) and peroxynitrite (ONOO<sup>-</sup>) (Dykes and Rooney, 2007). Thiol or sulfhydryl (SH) groups of proteins are composed of both intracellular and extracellular proteins and glutathione. They quench free radicals, and also take part in detoxification, signal transduction, apoptosis and other functions at the molecular level (Prakash et al., 2009).

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Phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) or InsP<sub>6</sub>) (PA) represents storage form of P in seeds. It typically represents 50%-85% of seed total P in wheat, and can be from one to several percent of seed dry weight (Khan et al., 2007). As a major pool in the flux of P through agroecosystems it represents a sum equivalent to > 50% of all P fertilizer used annually (Raboy, 2009). Nutrition rich in PA can substantially decrease micronutrients absorption as Ca, Mg, Fe, Mn, Zn, Cu, and Co due to phytates (PA salts) excretion by human and non-ruminant animals as poultry, swine, and fish (Doria et al., 2009). They have in common the lack of the ability to digest and utilize PA, thus representing antinutrient for them. It can cause micronutrients deficiencies leading to anemia, tissues hypoxia, heart failure, insufficient immune competence, impaired fine motor skills and memory capacity, growth retardation, impaired reproductive performance and difficulties in parturition, especially for populations in developing countries and people with inadequate nutrition (Reichwald and Hatzack, 2008).

Climate is a complex of factors that affects quality of agricultural products by combined action causing Genotype × Environment interaction (GEI). Changes of temperature and precipitation levels affect physiological processes in plants (Olesen et al., 2011). Modeling GEI by climatic variables can be considered as a predictive strategy, and relevant information obtained can be implemented in geographic databases, with ecological data applied to a representative time scale (Voltas et al., 2005). The objective of this study was to quantify the effects of environment (E), genotype (G), and their interaction (GEI) on the examined traits and also by the use of the factorial regression, the influence of climatic factors on the GEI for antioxidants and phytic acid contents in bread and durum wheat from multi-environment trial.

## MATERIAL AND METHODS

### Plant material and experimental design

The plant material used in this research consisted of 15 bread wheat (*Triticum aestivum* L. subsp. *aestivum*) and 15 durum wheat (*Triticum durum* Desf.) genotypes. This genetic material was selected from the GeneBank of Institute of Field and Vegetable Crops in Novi Sad and from the GeneBank of the Maize Research Institute "Zemun Polje" in Belgrade, Serbia. The cultivars and breeding lines of bread wheat were of diverse origin-from Serbia (SER), Croatia (CRO), USA, France (FRA), Macedonia (MAC), and Austria (AUS) and consisted of: 'Žitarka' (CRO), 'Stephens' (USA), 'Renan' (FRA), 'Caldwell' (USA), 'Abe' (USA), 'Auburn' (USA), 'Frankenmuth' (USA), 'Apache' (FRA), 'ZP AU 12' (MAC), 'Marija' (CRO), '87/Ip' (SER), 'Tecumseh' (USA), 'Pobeda' (SER), 'Zemunski rosa' (SER), and 'Ludwig' (AUS).

The cultivars and breeding lines of durum wheat belonged to International Maize and Wheat Improvement Center (CIMMYT); from the 37<sup>th</sup> Elite Durum Unreplicated Yield Trial (37EDUYT) and to International Center for Agricultural Research in the Dry Areas (ICARDA) from the Durum Segregating Populations – Mediterranean Dryland (DSP-MD-01; season 2000-2001), and also from Serbia (ZP10/I and ZP34/I), Slovakia (SOD 55), and Italy (Varano).

The trials were sown at the three test sites in Serbia: Rimski Šančevi (RS; 45°19'51" N, 19°50'59" E), Zemun Polje (ZP; 44°52' N, 20°19' E), and Padinska Skela (PS; 44°57' N, 20°26' E) during two growing seasons 2010-2011(11) and 2011-2012(12). The experimental design was randomized complete block design (RCBD) with four replicates. The experimental plot consisted of five rows of 1 m length with 0.2 m inter-row spacing. The elementary plot consisted of three internal rows of 0.6 m<sup>2</sup> area (3 × 0.2 m × 1 m) and was used for analysis. Haplic Chernozem (CHha) is the soil at RS and ZP sites and Humic Gleysol (GLhu) at PS (IUSS Working Group WRB, 2006). The mineral fertilizers (NPK 15:15:15, MAP) were applied before seeding according to the recommendation based on the analysis of soil chemical properties and available content of P, K, and mineral N reserves. Seeds anti-fungal protection was achieved with difenoconazole (3-chloro-4-[(2*RS*,4*RS*;2*RS*,4*SR*)-4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether; 30 g L<sup>-1</sup> Dividend 0.30 FS, Syngenta, Basel, Switzerland) in 2010-2011 season, and tebuconazole ((*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol; 60 g L<sup>-1</sup> Raxil 0.60 FS, Bayer CropScience, Monheim am Rhein, Germany) in 2011-2012 season. Sowing was done mechanically at the RS and by hand at the PS and the ZP. In the spring top dressing consisted of fertilizers-urea (CO(NH<sub>2</sub>)<sub>2</sub>, 46% N) (at the PS11, PS12, ZP12), calcium ammonium nitrate-CAN (5Ca(NO<sub>3</sub>)<sub>2</sub>NH<sub>4</sub>NO<sub>3</sub>·10H<sub>2</sub>O, 27% N) (ZP11), and ammonium nitrate-AN (NH<sub>4</sub>NO<sub>3</sub>, 34% N) (RS11, ZP11, RS12) application. Integral protection against pests and weeds was accomplished by the proper use of adequate pesticides and its efficacy was monitored, and crop damages were avoided.

### Climatic conditions during vegetation seasons

Climatic factors were measured at the field sites during March-June of the growing period and were provided by the Hydro-meteorological Service of Serbia and the PKB Agroekonomik Institute (Padinska Skela). Maximum temperature (mxt, °C), minimum temperature (mnt, °C), mean temperature (mt, °C), relative humidity (rh, %), sunshine hours (sh, h), and precipitation (pr, mm) mean values for March (1), April (2), May (3), and June (4) were recorded. The winter moisture reserves (wmr, mm), representing sum of daily precipitation for the period November-February was also calculated. Climatic data

are presented in Table 1, which were previously published in Branković et al. (2014).

### Chemical traits analyses

Measured chemical-technological traits contents were determined spectrophotometrically (UV-1601 spectrophotometer, Shimadzu Corporation, Kyoto, Japan): phytic acid (PA) determined by the method Dragičević et al. (2011); inorganic P ( $P_i$ ), determined also by Dragičević et al. (2011); total yellow pigment by American Association of Cereal Chemists (AACC, 1995) method; total soluble phenolic compounds by the method of Simić et al. (2004) and the content was expressed in  $\mu\text{g}$  ferulic acid equivalent (FAE); free protein sulfhydryl groups (PSH) by the method of de Kok et al. (1981). Analytes were expressed on DM basis. The phytic acid P ( $P_p$ ) content was obtained by dividing the value of phytic acid content by a factor of 3.55 (Barac et al., 2006). For these analyses grains were grinded in laboratory mill (Laboratory Mill 120, Perten Instruments, Hågersten, Sweden) (particles size < 500  $\mu\text{m}$ ).

### Statistical analyses

For the analysis of the chemical traits two-way fixed combined ANOVA, based on randomized complete block design (RCBD), with the effects of genotype

and environment as fixed ones, was used. Environment represented Year  $\times$  Test site combination. The significance of chemical traits means differences for environments was tested using the Tukey (HSD) test. ANOVA and Tukey (HSD) test were performed by the use of the STATISTICA 9.0 (StatSoft, Tulsa, Oklahoma, USA). Testing the difference in trait means between bread and durum wheat was carried out using *t*-test. The multiple factorial regressions (van Eeuwijk et al., 1996) following “forward” procedure of climatic variables inclusion for the explanation of GEI for PA and antioxidants contents of tested genotypes were used. Data analysis was done within R computing environment (R Development Core Team, 2010).

## RESULTS AND DISCUSSION

The GEI has three adverse effects in plant breeding: i) It reduces the correlation between genotypic and phenotypic values, decreasing the progress from the selection and making the selection of superior and stable genotypes in a wide range of environments difficult; ii) as a component of a trait phenotypic variance, it decreases heritability and hinders breeding for complex traits; and iii) it also masks the potential benefits of exotic materials introgression (Fan et al., 2007). However for specific selection being achieved GEI will help select genotypes for each environment. In order to assess the biological basis of interaction an analysis often includes additional information (climatic covariates), and the models most frequently applied are: partial least squares regression (Aastveit and Martens, 1986) and factorial regression (van Eeuwijk et al., 1996). The effect of seasonal variability on wheat yield is well studied (Lobell and Field, 2007; You et al., 2009), but less information exists regarding climate impact on wheat grain antioxidants and PA contents. The potential for good wheat yield and quality is initiated during vegetative development stages (Branković, 2014). Spikes formation, grain filling, and ripening are the most important phases of wheat development, affecting final quality of the grain, and climatic conditions in these stages have the greatest impact on quality traits (Marta et al., 2011).

Mean values for PA content differed across all six environments significantly ( $P < 0.05$ ) for bread wheat, but for durum wheat difference between ZP12 and PS12 was nonsignificant ( $P > 0.05$ ) (Table 2). Although the total mean difference was nonsignificant between bread and durum wheat ( $P > 0.05$ ), it was significant ( $P < 0.05$ ) at the RS11, RS12, and ZP12 (Table 2). Based on the ANOVA the major source of variation for PA content was GEI, G afterwards, and then E for bread wheat and E, GEI, and G, in descending order, for durum wheat (Table 3). GEI for PA was high (> 30% of the sum of squares-SS) in both, the bread and the durum wheat, but higher in bread for 21.67% compared to durum wheat. Models of climatic factors in the highest percentage (> 91%) were useful in

**Table 1. Climatic conditions during two growing seasons (2010-2011 and 2011-2012) at the test-environments.**

Month	Environments					
	RS11	PS11	ZP11	RS12	PS12	ZP12
Maximum temperature (°C)						
March	11.0	11.7	11.9	15.6	15.6	15.4
April	18.9	18.9	19.1	18.9	19.7	19.3
May	22.6	23.3	22.6	22.6	23.9	22.7
June	26.8	27.5	27.3	29.3	30.6	29.9
Minimum temperature (°C)						
March	1.2	0.3	2.2	1.2	-0.5	1.7
April	7.6	4.5	7.6	7.2	4.0	7.7
May	10.9	7.1	11.3	11.7	7.9	11.5
June	15.0	11.6	15.4	15.8	11.1	16.4
Mean temperature (°C)						
March	5.7	5.7	8.0	8.1	7.7	8.9
April	13.2	12.1	14.4	13.0	12.4	13.5
May	16.8	15.4	17.5	17.4	16.0	17.0
June	20.9	19.9	22.2	22.9	21.7	24.3
Relative humidity (%)						
March	77.6	79.4	70.2	55.4	60.9	55.3
April	62.6	67.8	58.5	68.5	72.8	65.2
May	72.7	80.1	68.5	70.4	79.8	70.6
June	69.2	77.7	63.3	61.7	72.1	56.4
Precipitation sum (mm)						
March	26.2	21.6	18.6	4.1	1.6	2.5
April	22.8	25.8	14.1	82.8	63.0	73.3
May	63.0	90.0	94.8	52.2	72.0	81.8
June	36.9	41.4	23.0	27.5	15.0	16.1
Sunshine hours sum (h)						
March	159.6	162.0	103.9	241.4	253.9	234.9
April	205.9	222.1	191.2	204.2	209.2	145.3
May	269.5	255.9	244.5	253.4	230.7	199.5
June	284.5	280.3	257.6	359.0	344.3	313.5
Other variables						
wmr, mm	170.7	171.6	194.7	163.0	113.6	172.3

RS: Rimski Šančevi; PS: Padinska Skela; ZP: Zemun Polje; 11: 2010-2011 growing season; 12: 2011-2012 growing season; wmr: winter moisture reserves (sum of daily precipitation for November-February period).



**Table 2. Content of phytic acid (PA), inorganic P (P<sub>i</sub>), phytic acid P/inorganic P (P<sub>p</sub>/P<sub>i</sub>), total yellow pigment, total soluble phenols, and free protein sulphhydryl groups (PSH) across six test-environments.**

Chemical trait content	Wheat species	Environments						
		RS11	ZP11	PS11	RS12	ZP12	PS12	Mean
PA, mg g <sup>-1</sup>	Bread	17.17 ± 1.19aA	15.46 ± 1.26fA	15.65 ± 1.53eA	15.52 ± 2.29dA	15.92 ± 0.98cA	16.56 ± 1.10bA	16.05a
	Durum	16.47 ± 0.63bB	15.48 ± 0.66dA	16.18 ± 1.32cA	13.74 ± 1.02eB	16.79 ± 1.08aB	16.79 ± 1.12aA	15.91a
P <sub>i</sub> , mg g <sup>-1</sup>	Bread	0.38 ± 0.05eA	0.51 ± 0.09aA	0.46 ± 0.06bA	0.36 ± 0.05fA	0.41 ± 0.07dA	0.42 ± 0.06cA	0.42a
	Durum	0.35 ± 0.04eA	0.48 ± 0.07aA	0.40 ± 0.05dB	0.44 ± 0.06bB	0.42 ± 0.04cA	0.39 ± 0.05dA	0.41a
P <sub>p</sub> /P <sub>i</sub>	Bread	12.86 ± 1.27aA	8.78 ± 1.41eA	9.67 ± 0.92dA	12.04 ± 1.04bA	11.26 ± 2.16cA	11.33 ± 1.23cA	10.99a
	Durum	13.33 ± 1.61aA	9.29 ± 1.48eA	11.74 ± 2.25cB	8.92 ± 1.27fB	11.48 ± 1.38dA	12.30 ± 1.54bB	11.18a
Total yellow pigment, µg βCE g <sup>-1</sup>	Bread	3.98 ± 0.53baA	3.92 ± 0.68bcA	4.07 ± 0.56aA	3.87 ± 0.59dcA	3.81 ± 0.75dA	3.58 ± 0.63eA	3.87a
	Durum	4.20 ± 0.52cbA	4.14 ± 0.68cA	4.44 ± 0.74aA	4.28 ± 0.85bA	4.22 ± 0.66cbA	4.17 ± 0.65cB	4.24a
Total soluble phenols, µg FAE g <sup>-1</sup>	Bread	968.84 ± 220.52cA	897.95 ± 141.29eA	906.76 ± 151.03edA	907.69 ± 153.11dA	1180.01 ± 163.61bA	1278.30 ± 210.92aA	1023.26a
	Durum	999.81 ± 111.13dA	775.93 ± 141.71fB	1056.55 ± 182.60bB	1187.77 ± 88.84aB	950.55 ± 92.42cB	1022.84 ± 125.52cB	998.91a
PSH, nmol g <sup>-1</sup>	Bread	37.32 ± 10.17eA	80.55 ± 30.33dA	125.65 ± 21.76aA	89.43 ± 9.50cA	112.82 ± 13.99bA	91.86 ± 16.07cA	89.61a
	Durum	50.09 ± 22.19eB	119.45 ± 38.26bB	133.75 ± 20.96aA	100.64 ± 24.47cA	100.98 ± 20.67cB	54.01 ± 20.55dB	93.15a

Means in each row with the same lower-case letter are not significantly different according to Tukey's test ( $P < 0.05$ ).

Means with same uppercase letter are not significantly different between wheat species according to the *t*-test ( $P < 0.05$ ).

RS: Rimski Šančevi; PS: Padinska Skela; ZP: Zemun Polje. 11: 2010-2011 growing season; 12: 2011-2012 growing season; βCE: β carotene equivalent; FAE: ferulic acid equivalent.

**Table 3. Two-way ANOVA for phytic acid (PA) and inorganic P (P<sub>i</sub>) contents and phytic acid P/inorganic P (P<sub>p</sub>/P<sub>i</sub>).**

Source of variations	df	PA			P <sub>i</sub>			P <sub>p</sub> /P <sub>i</sub>		
		SS	SS (%)	MS <sup>†</sup>	SS	SS (%)	MS <sup>†</sup>	SS	SS (%)	MS <sup>†</sup>
Bread wheat										
Environment (E)	5	105.74	15.6	21.15***	0.66	35.3	0.13***	698.02	50.0	139.60***
Genotype (G)	14	271.71	40.1	19.41***	0.92	49.2	0.07***	507.08	36.3	36.22***
G × E	70	300.62	44.3	4.29***	0.29	15.5	0.004***	192.08	13.7	2.74***
Error	252	11.92		0.047	0.017		0.000066	16.55		0.066
Durum wheat										
Environment (E)	5	335.30	51.2	67.03***	0.48	35.0	0.095***	896.43	48.6	179.28***
Genotype (G)	14	91.98	14.1	6.56***	0.52	38.0	0.037***	578.45	31.3	41.32***
G × E	70	227.35	34.7	3.25***	0.37	27.0	0.005***	371.42	20.1	5.31***
Error	252	11.67		0.046	0.018		0.00007	22.92		0.091

\*\*\*  $P < 0.001$ ; <sup>†</sup>tested against error mean square (MS).

SS: Sum of squares; df: degrees of freedom; MS: mean squares.

interpreting GEI for PA, and included rh in June, sh in April, mt in April, and wmr for genotypes of bread wheat, as well as pr in June and April, mxt in April and mt in June for durum wheat (Table 5).

The PA content in plants varies dependently on the stage of maturity, genotype, climatic factors, and location (Singh, 2008). Bueckert et al. (2011) described influence of temperature and precipitation on grain PA content in chickpea. The most important month with the highest percentage of interpreted interaction SS was April (Table 5) for both, bread and durum wheat, when stem elongation stage occurred, and when the plants grow most intensively. During this period leaf area was increased to five times in comparison to tillering, when organogenesis took place to form the definite number of flowers and their fertility (Denčić et al., 2012). According to Bohn et al. (2008) rain, drought, and high temperature during this developmental phase influence inositol phosphate kinases (Ipks) important for PA synthesis. In wheat, PA synthesis starts at flowering and it accumulates during grain development, until maturation and desiccation, so precipitation and high temperatures exhibit crucial role in this period (Bohn et al., 2008). Similarly, that was confirmed with the influence of pr, rh, and mt in June on PA content in our study. According to Li et al. (2013) six

eco-physiological factors had a significant impact on the PA content in soybean. These are soil factors (available K, P, N, and S) and meteorological factors (mean temperature during flowering and pods formation, mean temperature during grain filling and ripening). This is partly consistent with the results of this work, particularly in terms of the average daily temperature effect during grain filling. Singh et al. (2012) emphasized higher temperature and water-deficit conditions as causes of the maximum PA content in the wheat grains.

Across all six environments mean P<sub>i</sub> content was significantly different ( $P < 0.05$ ) for bread wheat, but nonsignificant difference existed between PS11 and PS12 for durum wheat (Table 2). The difference between bread and durum wheat P<sub>i</sub> content means was significant ( $P < 0.05$ ) only at the PS11 and RS12 environments, but not for total environmental mean (Table 2). ANOVA determined the same hierarchy of importance in sources of variation for the P<sub>i</sub> content in bread and durum wheat both:  $G > E > GEI$  (Table 3). The means difference for P<sub>p</sub>/P<sub>i</sub> was significant ( $P < 0.05$ ) among all environments for durum wheat and also among most environments for bread wheat, except between ZP12 and PS12 (Table 2). Also, significance ( $P < 0.05$ ) of means difference between bread and durum wheat was showed at the PS11, RS12,

and PS12 (Table 2). Similarly,  $P_p/P_i$  in bread and durum wheat, showed the descending order of influence:  $E > G > GEI$  (Table 3). GEI was moderately high (10-30%) for  $P_i$  content and  $P_p/P_i$  in bread and durum wheat (Table 3). In interpreting GEI for the  $P_i$  following significant models ( $> 92\%$  of SS of the interaction) were obtained: pr in May, mnt and mxt in April, mt in May for bread wheat, as well as pr in May, mnt in March, April and June for durum wheat (Table 5). According to Grabowski et al. (2014) average daily mean temperatures in the spring and summer months were inversely correlated with the wheat grain P content, what was confirmed in our study for  $P_i$  content in bread wheat in 2011-2012, when it was decreased 11.08% on average, in comparison to 2010-2011. They also found positive correlation between average minimum temperatures during vegetative developmental phases with the grain P content. Our data (Table 5) also emphasized the significance of minimum temperatures during March and April on the  $P_i$  content in bread and durum wheat. After wheat flowering, climatic factors primarily affect the grain size and its composition (Semenov et al., 2014). The optimum temperature for the longest wheat grain filling is 15-20 °C (Tomic et al., 2013). Dupont et al. (2006) found that drought reduces grain size by shortening the filling phase and the high temperature and drought jointly affect the duration of grain filling, rather than individually. To this regard, Wang et al. (2003) found that  $P_p$  content was significantly and positively correlated with initial grain filling rate, average rate of grain filling, and filling percentage, while  $P_i$  was negatively correlated with all of these indicators. In our study lower precipitation sums and higher temperatures in May and June, during flowering and grain filling periods, increased average  $P_i$  for 2011-2012 growing season in durum wheat. The largest percentage of the explained SS of the interaction for the  $P_i$  was obtained in May for bread wheat, and in April for durum wheat (Table 5). In May spikes formation was finished and flowering, fertilization, grain formation and grain filling followed. The highest percentage of the explained SS of the interaction ( $> 91\%$ ) for the  $P_p/P_i$  was obtained by the models involving: sh in April, pr in May, mt and pr in June for bread wheat, as well as the mxt in April, pr, mt and sh in May, for durum wheat (Table 5). The most important month for which the largest percentage of the SS of the interaction was obtained was April for bread wheat, and June for durum wheat (Table 5).

Most of the nonsignificant differences among environments for trait means were observed for yellow pigment content between four pairs of environments in bread wheat, and between seven in durum wheat (Table 2). The mean difference between bread and durum wheat was significant only at the PS12 (Table 2). Based on the ANOVA hierarchy of importance of the sources of variation influencing total yellow pigment was:  $G > GEI > E$  for both the bread and the durum wheat (Table 4).

This is consistent with the results of Clarke et al. (2006) for durum wheat in different growing conditions, while according to Hadži Tašković Šukalović et al. (2013) E was the most important in determining total yellow pigment in bread and durum wheat. GEI was moderately high in bread wheat and high in durum wheat. Models of climatic variables that significantly interpreted ( $> 95\%$ ) GEI for total yellow pigment content included: sh in May, mxt in April, and pr in May and March for bread wheat, as well as mt in March, April and May, and wmr for durum wheat (Table 5). March contributed the largest percentage of the SS of the GEI with its prevailing climatic variables for both bread and durum wheat (Table 5). According to Howitt and Pogson (2006) during wheat seed germination, higher yellow pigment content is correlated with an inhibition of peroxidase activity and promotion of seed germination. In our study climatic variables influencing GEI significantly were found for November-February period (wmr) and March, when the germination, seed emergence and early phases of vegetative growth occurred. Lukow et al. (2012) found that the bread wheat grown at the site with higher solar radiation had a higher content of carotenoids in the early stages of seed development, which acted as a temporary protective mechanism against photo-stress, until the permanent protective coating was formed. Clarke et al. (2006) found a weak correlation between the warm and wet growing seasons and yellow pigment content in durum wheat, and in our study increased content of total yellow pigment was confirmed in bread and durum wheat, both, in the 2010-2011, which had more precipitation in later phases of vegetation season and also less solar radiation. The retention of carotenoids into the mature grain stage was lower at the environments with the higher solar radiation and accordingly Asada (2006) proposed that carotenoids have been used for the ROS scavenging at the earlier stages of wheat development. Interestingly, similar results for total yellow pigment synthesis in terms of temperature and sunshine hours influence, showed Roselló et al. (2011) for tomato.

Mean values for total soluble phenolic compounds were not significantly different ( $P < 0.05$ ) between ZP11 and PS11 for bread wheat and between ZP11 and RS12 for durum wheat (Table 2). Most of significant mean difference ( $P < 0.05$ ) between bread and durum wheat was recorded for this trait at the five environments except RS11 (Table 2). ANOVA showed the impact of sources of variation for the total soluble phenolic compounds in the following descending order:  $E > G > GEI$  for bread wheat and  $E > GEI > G$  for durum wheat (Table 4), which is in agreement with results of other authors (Mpofu et al., 2006; Moore et al., 2006; Hadži Tašković Šukalović et al., 2013). GEI for total soluble phenolic compounds was moderately high in bread wheat, and high in durum wheat. The GEI for total soluble phenolic compounds was with the highest percentage of the SS ( $> 94\%$ ) explained by the models that included: pr and rh in March, mxt in April

**Table 4. Two-way ANOVA for antioxidants contents.**

Source of variations	df	Total yellow pigment			Total soluble phenols			Free protein sulfhydryl groups (PSH)		
		SS	SS (%)	MS <sup>†</sup>	SS	SS (%)	MS <sup>†</sup>	SS	SS (%)	MS <sup>†</sup>
Bread wheat										
Environment (E)	5	4.25	5.4	0.85***	6838473.42	43.4	1367694.68***	229880.90	70	45976.18***
Genotype (G)	14	53.66	69.0	3.83***	5737555.74	36.4	409825.41***	10020.16	3	715.72***
G × E	70	19.92	25.6	0.28***	3182430.66	20.2	45463.30***	88916.44	27	1270.23***
Error	252	1.35		0.016	210605.60		835.74	10647.44		42.25
Durum wheat										
Environment (E)	5	1.71	1.7	0.34***	4794336.49	49.3	958867.30***	285507.87	56.8	57101.57***
Genotype (G)	14	67.97	67.2	4.85***	1482042.50	15.2	105860.18***	74222.84	14.8	5301.63***
G × E	70	31.52	31.1	0.45***	3446433.13	35.5	49234.76***	142745.18	28.4	2039.22***
Error	252	1.44		0.017	155347.03		616.46	5598.42		22.22

\*\*\* $P < 0.001$ ; <sup>†</sup>tested against error mean square (MS).

SS: Sum of squares; df: degrees of freedom; MS: mean squares.

and pr in May for bread wheat, as well as mt in April, pr, rh and mt in May for durum wheat (Table 5). The most important month for the established interaction was April for both bread and durum wheat (Table 5). Precipitation and temperatures probably influenced specific phenolic precursors during March and April when tillering and stem elongation was underway in our investigation. It was found that the higher average monthly temperatures

and lower rainfall negatively affected the total phenolic compounds in bread and durum wheat (Feng et al., 2007). According to Yu et al. (2004) the number of hours with air temperature > 32 °C affected the antioxidant potential of bread wheat, what was also confirmed for the total phenolic compounds in bread wheat bran fraction by Moore et al. (2006). In May and June during 2010-2011, 4 to 8 d with temperatures greater than 30 °C were recorded,

**Table 5. Multiple factorial regressions of climatic variables explaining Genotype × Environment interaction for phytic acid, inorganic P, antioxidants contents, and phytic acid P/inorganic P.**

Model	Environmental variables included in the final model <sup>1</sup> for bread wheat	Residual	Environmental variables included in the final model for durum wheat	Residual
Phytic acid				
All variables	rh4 (36.2); sh2 (32.2); mt2 (14.0); wmr (9.4)	8.2	pr4 (35.6); pr3 (30.7); mxt2 (17.0); mt4 (9.6)	7.0
March	rh (30.7); pr (20.2); sh (18.4); mnt (17.1)	13.7	rh (31.7); mxt (23.4); pr (17.0); mnt (16.2)	11.7
April	pr (29.2); sh (26.5); mnt (23.9); mxt (11.2)	9.2	mxt (35.0); sh (22.3); pr (21.3); mnt (15.3)	6.1
May	mnt (33.8); pr (20.7); rh (19.9); sh (11.0)	14.6	pr (29.7); sh (24.1); mnt (22.0); mxt (14.9)	9.4
June	rh (36.2); mnt (23.2); sh (15.0); mnt (15.0)	10.7	pr (35.6); mxt (23.3); sh (17.7); mt (12.9)	10.5
Inorganic P				
All variables	pr3 (50.7); mnt2 (19.8); mxt2 (12.9); mt3 (11.4)	5.1	pr3 (39.2); mnt1 (21.6); mnt4 (18.5); mnt2 (13.0)	7.8
March	mxt (34.4); pr (23.5); sh (20.3); mnt (9.9)	11.9	rh (34.4); sh (28.4); mnt (19.6); mt (11.4)	6.2
April	mxt (25.4); mt (21.6); pr (18.5); sh (13.3)	21.2	pr (28.8); mxt (24.7); mt (20.6); sh (20.1)	5.9
May	pr (50.7); mnt (19.4); rh (14.4); mt (10.9)	4.6	pr (39.2); mxt (21.2); mt (13.2); sh (12.6)	13.8
June	mxt (29.5); sh (28.8); pr (14.2); mnt (8.3)	19.2	sh (34.0); mxt (23.3); mt (19.7); pr4 (12.7)	10.3
Phytic acid P/inorganic P				
All variables	sh2 (52.1); pr3 (14.7); mt4 (12.4); pr4 (12.4)	8.3	pr3 (34.2); mxt2 (27.8); mt3 (19.4); sh3 (11.7)	6.9
March	mxt (40.5); mt (18.6); rh (15.4); sh (11.0)	14.5	rh (25.6); pr (23.5); mnt (21.5); mxt (17.8)	11.6
April	sh (52.1); mxt (13.1); mnt (11.0); pr (10.4)	13.4	sh (25.9); mxt (25.5); pr (22.4); mt (14.4)	11.8
May	sh (35.6); mxt (14.8); mnt (13.5); pr (12.9)	23.3	pr (34.2); mxt (22.4); mt (21.6); mnt (12.1)	9.6
June	mt (28.2); sh (21.2); pr (15.1); mxt (12.3)	23.4	sh (32.1); pr (23.5); mnt (20.1); mxt (15.1)	9.1
Total yellow pigment				
All variables	sh3 (38.5); mxt2 (28.6); pr3 (14.9); pr1 (13.0)	5.0	mt3 (35.1); mt2 (25.6); wmr (17.6); mt1 (16.9)	4.7
March	mt (33.5); mnt (24.3); rh (16.9); sh (16.7)	8.7	mxt (28.2); rh (23.6); mnt (23.4); pr (20.1)	4.7
April	sh (36.7); mxt (28.5); pr (13.2); mt (5.8)	15.8	mt (31.0); pr (25.3); mxt (16.9); sh (9.7)	17.1
May	sh (38.5); mxt (25.6); pr (15.8); mt (10.4)	9.6	mt (35.1); pr (21.6); sh (14.9); mxt (10.9)	17.5
June	mt (33.7); pr (26.6); sh (16.1); mnt (11.0)	12.6	mnt (25.9); sh (25.7); rh (24.9); pr (18.0)	5.4
Total soluble phenols				
All variables	pr1 (27.8); rh1 (23.9); mxt2 (23.5); pr3 (20.1)	4.7	pr3 (26.0); mt2 (25.6); rh3 (23.7); mt3 (19.2)	5.5
March	mnt (41.6); rh (17.7); pr (16.2); mxt (11.1)	13.4	mnt (21.3); sh (19.7); mt (17.5); pr (14.6)	27.0
April	rh (29.0); mxt (23.5); pr (21.3); mnt (19.4)	6.9	pr (33.5); mt (23.2); rh (21.0); mnt (14.2)	8.1
May	mxt (19.0); mt (13.5); rh (11.1); sh (10.3)	46.1	pr (26.0) rh (21.5) mt (19.2) mnt (9.2)	24.1
June	mnt (26.1); rh (23.9); pr (21.5); mt (18.4)	10.2	mxt (30.7) sh (23.5) pr (14.8) mt (11.2)	19.9
Free protein sulfhydryl groups				
All variables	mt2 (39.4); sh1 (20.8); rh2 (20.4); mxt1 (13.3)	6.0	rh3 (59.6); mnt3 (14.5); mxt2 (12.3); sh3 (8.0)	5.6
March	mxt (31.9); sh (31.4); mt (14.6); rh (5.2)	16.8	mnt (58.9); mt (12.9); rh (11.8); sh (6.7)	9.7
April	mt (39.4); sh (20.0); rh (17.6); mnt (8.1)	14.9	mnt (54.3); mt (15.4); mxt (12.0); pr (9.7)	8.7
May	mt (26.8); mxt (26.3); mnt (26.1); rh (11.8)	8.9	rh (59.6); mnt (14.5); mxt (12.0); sh (7.3)	6.7
June	pr (23.2); mxt (22.8); sh (21.6); mnt (7.8)	24.6	mnt (52.8); mxt (11.0); pr (10.4); sh (10.2)	15.7

<sup>1</sup>Variable significance is tested against error mean square  $P < 0.01$ .

mxt: Average maximum temperature; mnt: average minimum temperature; mt: average mean temperature; pr: precipitation sum; rh: average relative humidity; sh: sunshine hours sum; winter moisture reserves (wmr): daily precipitation sum for November-February period; 1: March; 2: April; 3: May; 4: June.

All reported values are given as a percentage of sum of squares of explained variance of Genotype × Environment interaction by the term.



while during the 2011-2012 it was 17-23 d depending on the site. Such high temperatures distribution caused increase in the total soluble phenolic compounds in bread wheat for 17.6% and in durum wheat for 10.4% in 2011-2012 in comparison to 2010-2011. Stracke et al. (2009) concluded that climatic factors have a greater impact on the carotenoids and phenolic acids concentrations in wheat grain than production methods (organic vs. conventional). The influence of temperature stress on antioxidants in bread wheat bran, especially the phenols, was recorded in Zhou and Yu (2004).

Significant ( $P < 0.05$ ) differences for PSH content were observed across most of the environments except between RS12 and PS12 for bread wheat, and between RS12 and ZP12 for durum wheat (Table 2). Although the total mean difference was nonsignificant between bread and durum wheat ( $P > 0.05$ ), it was significant ( $P < 0.05$ ) at the PS11 and RS12 (Table 2). ANOVA for PSH content determined order of influence of sources of variation, which was identical for both bread and durum wheat:  $E > GEI > G$  (Table 4). GEI for the PSH content was moderately high for both bread and durum wheat. Models consisting of climatic variables, with the highest percentage of explained SS of the interaction ( $> 94\%$ ) for the PSH content were: mxt and sh in March, mt and rh in April, for bread wheat, as well as mxt in April, rh, sh and mnt in May for durum wheat (Table 5). According to Kocsy et al. (2002) glutathione synthesis is induced not only by low, but also by high temperature what is similar to our results for both bread and durum wheat. Mobilization of reserves from crop stem is dependent on sink strength, which varies with the genotype and is affected by the water availability and climatic factors (Chaves and Oliveira, 2004). Modeling interaction using climatic variables by months underlined that the highest percentage of the SS of the interaction was obtained for May, for both bread and durum wheat (Table 5).

## CONCLUSION

Our results showed that Genotype  $\times$  Environment interaction (GEI) prevailed as source of variation for phytic acid (PA) and free protein sulfhydryl groups (PSH) in both bread and durum wheat, and for total soluble phenolic compounds in durum wheat. The major contribution to GEI represented climatic variables occurring during stages of stem elongation for PA and total soluble phenolic compounds and also flowering, fertilization, grain formation, and grain filling for PSH content. Generally, bread wheat genotypes showed better stability (smaller GEI and environment effects) for the content of the PA, inorganic P ( $P_i$ ), yellow pigment, and total soluble phenolic compounds, than durum wheat genotypes. Greater success of breeding would be expected for total yellow pigment and  $P_i$  contents in both bread and durum wheat, as genotype dominated over environment

and GEI. Models of climatic variables with joint action were obtained for efficient GEI explanation ( $> 92\%$ ) for PA and antioxidants contents, offering possibilities in the prediction of these quality traits in relation to more variable and changeable climate.

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